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Short communication

Synthesis, characterization and in vitro antibacterial activity of thiourea and urea derivatives of steroids

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Abstract

Some heterocyclic systems namely stigmest-6-en-7, 5α thiourea and stigmest-6-en-7, 5α urea derivatives of steroids were synthesized by the reaction of stigmest-5-en-7 one with thiourea/urea in the presence of a few drops of conc. HCl at 80 °C in high yield. All the compounds have been characterized by means of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic data. The antibacterial activity was first tested in vitro by the disk diffusion assay against two Gram-positive and two Gram-negative bacteria, and then the minimum inhibitory concentration (MIC) of compounds were determined. The results showed that steroidal thiourea derivatives inhibit growth as compared to steroidal urea derivatives of both type of the bacteria (Gram-positive and Gram-negative). Compounds **3** and **4** are better antibacterial agents as compared to standard drug chloramphenicol.

Keywords: Thiourea; Urea; Antibacterial activity

1. Introduction

Parasitic bacteria (Gram-positive and Gram-negative) continue to beleaguer and kill millions of people in the subtropical regions of the world. Sixty million people are infected and 20,000 deaths every year due to caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium* and *Escherichia coli*. These bacteria causes food poisoning, rheumatic, salmonellosis and diarrhea. Moreover drug resistance in food poisoning, rheumatic, salmonellosis and diarrhea can be attributed to the use of drugs (amoxicillin, norfloxacin, ciprofloxacin chloramphenicol) for treatment and to the adaptation of the bacterial parasite by developing alternate pathways for survival. Hence, the present strategy for new drug development is directed towards developing new steroidal molecules to inhibit the growth of parasite [1,2]. The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry. It is well known that a number of heterocyclic compounds containing nitrogen and sulphur exhibited a wide variety of biological activity. Thiourea and urea have been used as purification agents for the effluent of organic and inorganic, industrial, agricultural and mining wastes [3]. These compounds were also known to possess antidiabetic activity [4]. Thiourea, azacyclic urea, benzene sulfonyl urea, dihydropyrimidine thiones, thiazines, cyclic triazinanes pyralinones ester of imidazolidinones and their related analogues have become the focus of interest in recent past on account of their pharmacological activities. Thiazoles and their ester possess depressant activity with central nervous system [5], whereas urea and thiourea have been studied for the systematic control of tuberculosis [6–9] as well as anti-inflammatory activity during corrageenin induced edema in rats [10]. A few compounds of urea and thiourea (2-amino-1,3-thiazines) have shown bactericidal, fungicidal, herbicidal and algeacidal activities [11,12]. These compounds are useful in agriculture, spinning mixtures, paper and paints [13]. It has been described that acyl urea derivatives act as cation surfactants [14], while *S*-[*w*-(carboxamidino) alkyl] isothiourea was used as radiation protector in mammals' skin

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[15]. The compounds of urea and thiourea have exercised as wrinkle proofing agents for cotton and cotton polyester fabrics [16,17]. The utility of urea and thiourea derivatives were found in the preparation of triazines, isoxazoles and oxazoles. Triazines showed inhibitory activity against *Bacillus subtiles* and *Candida albicans* [18]. A series of diaryl substituted heterocyclic urea which were found to inhibit cholesterol O-acy1 transferase (ACAT) as hypocholestrolemic agents' in vitro and in vivo studies [19], but *N*, *N*′-disubstituted cyclic urea-3-benzamide was found to be HIV protease inhibitor; which may be useful in the treatment of AIDS [20]. Urea and thiourea compounds also could be used for elimination or detoxification of super antigens from body fluids [21–23], and for the treatment of haemoglobinopathies in the cases of sickle-cell anemia and β-thalassemia [24]. Steroidal compounds dramatically increase the diversity of certain biological properties [25–28]. In this paper the steroidal thiourea and urea derivatives have been synthesized by the condensation of the steroidal ketones with thioure/urea in the presence of a few drops of conc. HCl. The activities of these compounds were screened in vitro against *bacteria*.

2. Results and discussion

Steroidal thiourea/urea derivatives gave an yield of 75–90%. All the steroidal ketones were prepared by the reported method [29]. The steroidal ketone derivatives were used as starting materials for the preparation of thiourea/urea derivatives of steroid. The thiourea and urea derivatives of steroid were synthesized by the literature procedure [30] as indicated in Schemes 1 and 2. All the compounds were soluble in DMSO and ethanol. The structures of all the compounds were established by means of their IR, ^{1}H NMR, ^{13}C NMR, FAB mass spectra and the elemental analysis were carried out to check the purity of the compounds.

2.1. IR spectral studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of the cyclized thiourea and urea derivative analogues. The IR spectra of all the compounds showed ν(N–H) stretch at 3428–3448 cm^{-1} due to the ring closure. In addition, the absorption band at 1102–1155 cm^{-1} was attributed to the ν(C–N) stretch vibrations. The compounds of thiourea derivative showed intense bands at 1183–1206 cm^{-1} due to ν(C=S) stretch, urea derivative showed carbonyl amide group stretch at 1648–1672 cm^{-1}.

2.2. Nuclear magnetic resonance spectral studies

Further evidence for the formation of the compounds was obtained from ^{1}H NMR and ^{13}C NMR spectra, which provide diagnostic tools for the positional elucidation of the protons. Assignments of the signals are based on the chemical shifts and intensity patterns. The ^{1}H NMR spectrum of all the compounds showed singlet (exchangable with D_2O) for two ($2 \times$ NH) protons at δ (6.10–6.26), another singlet for one (C6-vinylic) proton at δ (5.4–5.8), a broad multiplet peak of all the acetoxy derivative compounds for C3α-proton at δ (5.06–5.2) and all the chloro derivative compounds for C3α-proton at δ (3.76–3.81). Thus on the basis of the above data the products have been characterized as steroidal thiourea and urea derivatives.

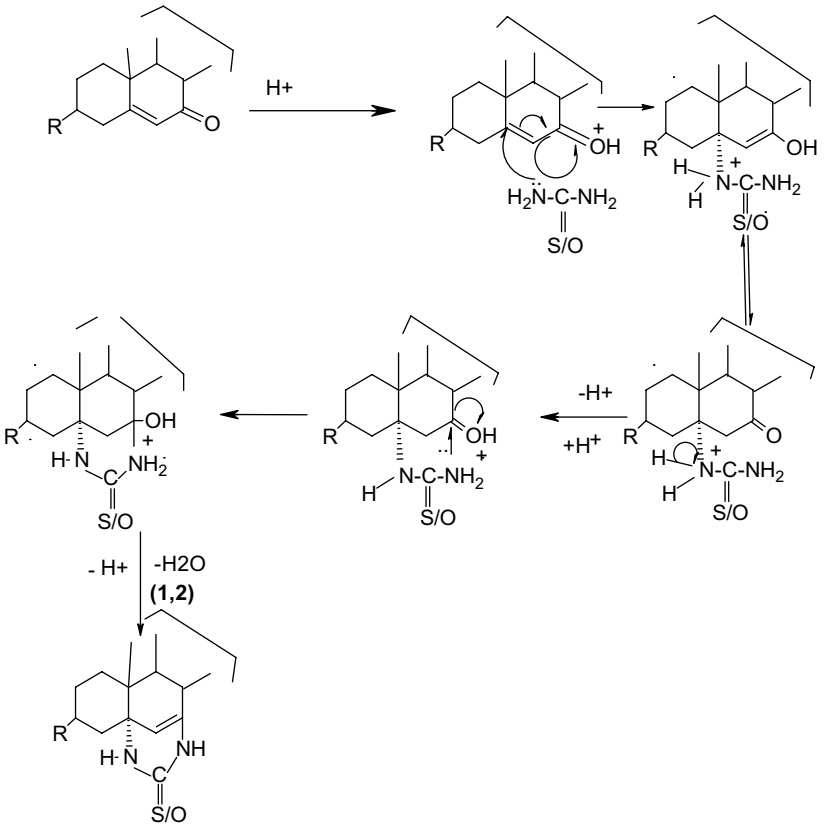
^{13}C NMR spectra of all the compounds were taken in $CDCl_3$ and the signal obtained further confirmed the proposed structures. All the compounds showed a signal at (136.45–154.52) ppm due to (C7–NH) of thiourea/urea cyclization. Thiocarbamoyl carbon (C=S) displayed a signal at (172.22–184.32) ppm of thiourea derivatives. Urea derivative showed a signal at (148.45–152.62) ppm due to carbonyl amide group. The characteristic peaks observed within the ^{13}C NMR spectra of thiourea/urea derivatives are given in Section 4.

2.3. FAB mass analysis

Characteristic peaks were observed in the mass spectra of compounds **3**–**6** which followed the similar fragmentation pattern. The spectrum of compound **3** showed a molecular ion peak ($M^{+\bullet}$) at *m/z* 526, compound **4** showed a molecular ion peak ($M^{+\bullet}$) at *m/z* 502/504, compound **5** showed a molecular ion peak ($M^{+\bullet}$) at *m/z* 510 and compound **6** showed a molecular ion peak ($M^{+\bullet}$) at *m/z* 486/488, urea derivative showed carbonyl amide group. The characteristic peaks observed within the mass spectra of thiourea/urea derivatives are given in Section 4.

2.4. In vitro antibacterial activity

The in vitro antibacterial activities of steroidal thiourea/urea (**3**–**6**) derivatives and cholesterol (**a**) were carried out using the culture of *S. aureus*, *S. pyogenes*, *S. typhimurium*, and *E. coli* by the disk diffusion method [31] and then the minimum inhibitory concentration (MIC) of all the compounds was determined. Choramphenicol (30 μg) was used as the standard drug, whereas DMSO poured disk was used as negative control. The minimum inhibitory concentration (MIC) was evaluated by the macrodilution test using standard inoculums of 10^{-5} CFL mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg/mL to each tube was added 100 μL of a 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C. Compounds **3** and **5** have 3β-acetoxy, and compounds **4** and **6** have 3β-chloro groups, respectively. The in vitro study results showed that the compounds chloro and acetoxy derivatives of steroidal thiourea were found to be the most active among all the compounds. The susceptibility of bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Table 1 reports the inhibition zones (mm) of each compound and these compounds were further checked by MIC



Scheme 1. Schematic diagram showing the mechanism of the synthesis of compounds **3**–**6**.

method. The results are presented in Table 2. The molecular structure of these active compounds showed enhanced activity. The distinct difference in the antibacterial property of these compounds further justifies the purpose of this study. The importance of such work lies in the possibility that the new compound might be more effective against bacteria for which a thorough investigation regarding the structure–activity relationship, toxicity and in their biological effects which would be helpful in designing more potent antibacterial agents for therapeutic use is required.

3. Conclusion

This research examined the antibacterial activities of new cyclized steroidal thiourea and urea derivatives prepared by the reaction of steroidal ketones with thiourea/urea in the presence of a few drops of HCl at 80 °C. The results showed that among all the four compounds, thiourea derivatives (**3**, **5**) are better antibacterial agents as compared to standard drug chloramphenicol.

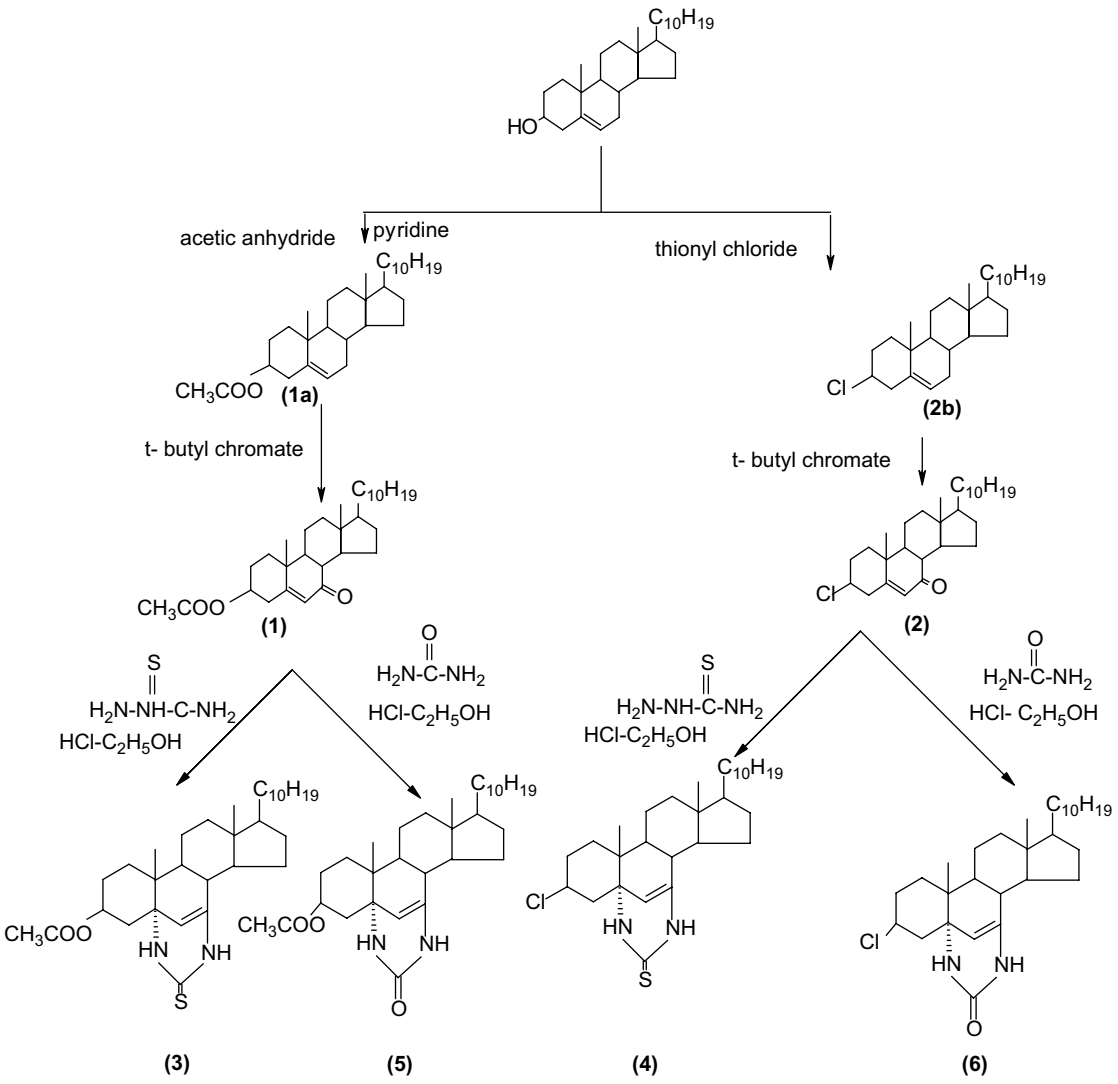
4. Experimental

4.1. Materials and methods

All the chemicals were purchased from Aldrich Chemical Company (U.S.A.) and were used without further purification. The reactions were monitored by precoated aluminium silica gel 60F 254 thin layer plates procured from Merck (Germany). All melting points were measured with a capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ^{1}H NMR ^{13}C NMR and mass spectrometry. IR spectra were recorded in KBr on a Perkin–Elmer model 1620 FTIR spectrophotometer. ^{1}H NMR spectra were recorded at ambient temperature using a Brucker spectroscopin DPX-300 MHz spectrophotometer in $CDCl_3$ and DMSO. The following abbreviations were used to indicate the peak multiplicity: s-singlet, d-doublet, t-triplet, m-multiplet. FAB mass spectra were recorded on a JEOL SX102 mass spectrometer using Argon/Xenon (6 kV, 10 mB gas). Column chromatography was performed on silica gel (Merck). Anhydrous sodium sulphate was used as a drying agent for the organic phase. 3β-Acetoxy-stegmest-5-en-7-one and 3β-chloro-stegmest-5-en-7-one were prepared according to published methods.

*4.2. 3β-Acetoxy-stigmest-6-en-7, 5α-thiourea (**3**)*

To a solution of ketone (**1**) (1.0 g) (2.13 mmol) in absolute ethanol (30 mL) a few drops of conc. HCl were added followed by thiourea (0.174 g, 2.30 mmol) and the mixture was refluxed for 1 h. After completion of the reaction the solvents were removed under reduced pressure and the residue was taken in the ether. The ethereal solution was washed successively with water, $NaHCO_3$ aq. solution (5%) and water, and dried over anhydrous sodium sulphate. Removal of solvents gave the crude product which was recrystallized from



Scheme 2. Schematic diagram showing the synthesis of compounds **3**–**6**.

methanol to give 3β-acetoxy-stigmest-6-en-7, 5α-thiourea. White solid; yield: 93%; m.p. 98 °C; Anal. Calc. for $C_{32}H_{50}N_2O_2S$: C, 73.00, H, 9.5, N, 5.32; found: C, 72, H, 9.5, N, 5.32; IR (KBr) ν_{max} cm^{-1}: 3430 (N–H), 1735 ($OCOCH_3$), 1636 (C=C), 1206 (C=S), 1155 (C–N); ^1H NMR (DMSO)/ppm: 6.10 (2H, s, 2 × NH, exchangeable with D_2O), 5.4 (1H, s, C6–H), 5.06 (H, m, $w1/2 = 17$ Hz axial, C3α–H), 2.15 (3H, s, $OCOCH_3$), 1.16 (C10–CH_3), 0.71 (C13–CH_3) 0.97 and 0.89 (remaining methyl protons); ^{13}C NMR ($CDCl_3$)/ppm: 172.22 (C=S), 152.28 (C7–NH), 108.70 (C=C), 67.26 (CH–O), 24.26 (CH_3–CO); FAB MS: *m/z* 526 (M + 1), 482 (M − CS), 468 (M − AcO), 467 (M − CSNH), 452 (M − CSN_2H_2), 387 (M − $C_{10}H_{19}$).

Table 1
Antibacterial activity of steroidal thiourea and urea derivatives

Compound	Corresponding effect on microoganisms			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
(**a**)	9.2 ± 0.2	7.8 ± 0.3	8.2 ± 0.2	9.0 ± 0.2
3	22.5 ± 0.5	21.6 ± 0.7	19.4 ± 0.3	23.4 ± 0.4
4	19.7 ± 1.2	23.2 ± 0.4	18.7 ± 0.9	20.7 ± 0.5
5	14.4 ± 0.5	12.4 ± 0.5	15.2 ± 0.5	16.3 ± 0.4
6	16.8 ± 1.4	15.2 ± 0.4	14.4 ± 0.8	14.8 ± 1.2
Choramphenicol	21.0 ± 0.5	22.2 ± 0.4	25.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

Compound (**a**) is cholesterol. Positive control (choramphenicol) and negative control (DMSO) measured by the Halo Zone Test (unit, mm).

Table 2
Minimum inhibition concentration (MIC) of steroidal thiourea and urea derivatives, positive choramphenicol control

MIC (μg mL^{-1})	Compound					Positive control
Strain	(**a**)	**3**	**4**	**5**	**6**	
S. aureus	512	32	64	64	64	32
S. pyogenes	512	64	64	128	128	32
S. typhimurium	512	32	32	128	128	32
E. coli	512	32	32	64	128	32

Compound (**a**) is cholesterol.

4.3. 3β-Chloro-stigmest-6-en-7, 5α thiourea (**4**)

To a solution of ketone (**2**) (1.0 g, 2.33 mmol) in absolute ethanol (30 mL) a few drops of conc. HCl were added followed by thiourea (0.189 g, 2.49 mmol) and the mixture was refluxed for 1 h. After completion of reaction the reaction mixture was usually worked up. Removal of solvents gave the crude product, which was purified by recrystallization from methanol to give 3β-chlorostigmest-6-en-7, 5α thiourea. Brown solid; yield: 89%; m.p.112 °C; Anal. Calc. for $C_{30}H_{47}SClN_2$: C, 71.64, H, 9.35, N, 5.57; found: C, 71.60, H, 9.36, N, 5.52; IR (KBr) ν_{max} cm^{-1}: 3438 (NH), 1646 (C═C), 1183 (C═S), 1102 (C−N), 705 (C−Cl); ^{1}H NMR (DMSO)/ppm: 6.26 (2H, s, 2 × NH, exchangeable with D_2O), 5.6 (1H, s, C6−H), 3.76 (1H, m, $w1/2 = 15$ Hz axial C3α−H), 1.18 (C10−CH_3), 0.77 (C13−CH_3), 0.99 and 0.84 (remaining methyl protons); ^{13}C NMR ($CDCl_3$)/ppm: 184.32 (C═S), 154.52 (C7−NH), 105.23 (C═C), 55.80 (C−Cl); FAB MS: *m/z* 502/504 (M + 1), 467 (M − HCl), 458/460 (M − CS), 443/445 (M − CSNH), 429/431 (M − CSN_2H_2), 363 (M − $C_{10}H_{19}$).

4.4. 3β-Acetoxystigmest-6-en-7, 5α-urea (**5**)

To a solution of ketone (**1**) (1.0 g, 2.13 mmol) in absolute ethanol (30 mL) a few drops of conc. HCl were added followed by urea (0.138 g, 2.30 mmol) and the mixture was refluxed for 2.5 h. After completion of the reaction the solvents were removed under reduced pressure and the residue was taken in ether. The ethereal solution was washed successively with water, aq. $NaHCO_3$ solution (5%) and water, and dried over anhydrous sodium sulphate. Evaporation of the solvents provided a crude solid product which was purified by recrystallization from methanol to give 3β-acetoxystigmest-6-en-7, 5α-urea off-orange solid; yield: 88%; m.p. 104 °C; Anal. Calc. for $C_{32}H_{50}N_2O_3$: C, 75.29, H, 9.80, N, 5.49; found: C, 75.24, H, 9.62, N, 5.46; IR (KBr) ν_{max} cm^{-1}: 3428 (NH), 1735 (OCOCH_3), 1672 (amide carbonyl), 1624 (C═C), 1136 (C−N); ^{1}H NMR (DMSO)/ppm: 6.21 (2H, s, 2 × NH, exchangeable with D_2O), 5.8 (1H, s, C6−H), 5.2.(1H, m, $w1/2 = 16$ Hz, axial, C3α−H), 2.2 (s, 3H, OCOCH_3), 1.18 (C10−CH_3), 0.78 (C13−CH_3) 0.94 and 0.88 of other methyl protons; ^{13}C NMR ($CDCl_3$)/ppm: 148.45 (C═O), 136.45 (C7−NH), 106.26 (C═C), 66.25 (CH−O), 22.0 (CH_3−CO); FAB MS: *m/z* 510 (M + 1), 482 (M − CO), 467 (M − CONH), 452 (M − CON_2H_2), 371 (M − $C_{10}H_{19}$).

4.5. 3β-Chloro stigmest-6-en-7, 5α urea (**6**)

To a solution of ketone (**2**) (1.0 g, 2.24 mmol) in absolute ethanol (30 mL) a few drops of conc. HCl were added followed by urea (0.143 g, 2.39 mmol) and the reaction mixture was refluxed for 2 h. After completion of reaction the reaction mixture was usually worked up. Removal of the solvents gave the crude solid product which was purified by recrystallization from methanol to give 3β-chlorostigmest-6-en-7, 5α urea. Dark brown solid (DMSO); yield: 82%; m.p. 118 °C; Anal. Calc. for $C_{30}H_{47}N_2OCl$: C, 73.92, H, 9.45, N, 5.72; found: C, 72.82, H, 8.95, N, 5.52; IR (KBr) ν_{max} cm^{-1}: 3448 (NH), 1648 (amide carbonyl), 1620 (C═C), 1122 (C−N), 710 (C−Cl); ^{1}H NMR (DMSO)/ppm: 6.17 (2H, s, 2 × NH, exchangeable with D_2O), 5.5 (1H, s, C6−H), δ 3.81 (1H, m, $w1/2 = 14$ Hz, axial, C3α−H), 1.12 (C10−CH_3) and 0.74 (C13−CH_3), 1.04 and 0.83 remaining methyl protons; ^{13}C NMR ($CDCl_3$)/ppm: 152.62 (C═O), 141.45 (C7−NH), 107 (C═C), 59.06 (C−Cl); FAB MS: *m/z* 486/488 (M + 1), 458/460 (M − CO), 451 (M−Cl), 543/545 (M − CONH), 428/430 (M − CON_2H_2), 347 (M − $C_{10}H_{19}$).

4.6. Organism culture and in vitro screening

Antibacterial activity was done by the disk diffusion method with minor modifications. *S. aureus*, *S. pyogenes*, *S. typhimurium* and *E. coli* were subcultured in BHI medium and incubated for 18 h at 37 °C, and then the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1}: 10 μL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 °C and poured onto an agar plate in a laminar flow cabinet. Five paper disks (6.0 mm diameter) were fixed onto nutrient agar plate. One milligram of each test compound was dissolved in 100 μL of DMSO to prepare stock solution and from stock solution different concentrations 10, 20, 25, 50, and 100 μg/μL of each test compound were prepared. These compounds of different concentrations were poured over disk plate onto it. Choramphenicol (30 μg/disk) was used as standard drug (positive control). DMSO poured disk was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Table 1 reports the inhibition zones (mm) of each compound and the controls. The minimum inhibitory concentration (MIC) was evaluated by the macrodilution test using standard inoculums of 10^{-5} CFL mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg/mL. To each tube was added 100 μL of 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C, and the results are presented in Table 2. Tests use DMSO and chloramphenicol as negative and positive controls, respectively.

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